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The relationship between immunologic system and ferritin levels in patients with transfusion dependent thalassemia: a retrospective single-center study

Cem Selim

Department of Adult Hematology, Şanlıurfa Mehmet Akif İnan Training and Research Hospital, Şanlıurfa, Turkey

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Corresponding Author: Cem Selim, dr.cemselim@gmail.com

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ABSTRACT

Aims: Thalassemia is an inherited anemia characterized by reduced or absent expression of α- or ß-globulin genes.[1] Transfusion dependent thalassemia (TDT) has an increased risk for serious infections, suggesting that a fundamental defect.

Methods: The study included 61 patients with TDT between the ages of 18 and 45 who were followed up in the hematology clinic of our tertiary care center between 2018 and 2023. For the determination of mononuclear cell surface markers, peripheral blood was processed according to standard procedures, labeled with monoclonal antibodies specific for CD4, CD8, CD3, CD19, and CD56, and analyzed by cytofluorometric analysis with a FACScan flow cytometer (Sysmex UF 1600).

Results: In our study, CD4, CD8, CD3, CD19, and CD56 values, which are lymphocyte markers, were examined to evaluate the immune system of patients diagnosed with TDT, and it was observed that there was no significant correlation between these values and the annual infection frequency and mean ferritin values of the patients.

Conclusion: The mean age of the 61 patients included in the study was 24.83±6.02 years, and 33 were female and 28 were male. The mean ferritin value was 3150.88±2553.51 ng/ml, and transferrin saturation was 42.42±14.43. The mean number of transfusions per year was 15.39±1.90, and the mean number of detectable infections per year was 2.85±2.02.

Keywords: Transfusion dependent thalassemia, immunologic system, ferritin

INTRODUCTION

Thalassemia is an inherited anemia characterized by reduced or absent expression of α- or β-globulin genes.[1] It is prevalent in the Mediterranean region as well as in the Far East and equatorial countries in the world, and approximately over 40.000 new patients are diagnosed each year. Due to the high frequency of consanguineous marriages, approximately 300 babies with thalassemia are born in our country every year, and this constitutes an important problem.[2] The prevalence of thalassemia carriage in Turkey is reported to be 2%, and this rate varies regionally (0.6%±13.1%).[3] Beta thalassemia occurs as a result of various mutations (quantitative defect in globin chain synthesis), primarily in the genes encoding globin chains on chromosome 11, may be transfusion dependent (TDT), and is an autosomal recessive disease.[4]

TDT has an increased risk for serious infections, suggesting that a fundamental defect in host defenses is present, which may be associated with iron overload, chronic immune stimulation by repeated blood transfusions, splenectomy, and immunodeficiency. The effects of iron overload include reduced antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages, changes in T-lymphocyte subsets, and modification of lymphocyte distribution in different parts of the immune system.[5] Infections are a common complication of thalassemia (12-13%) and can be fatal.[6] Infections are one of the top two causes of mortality in thalassemia, along with heart failure.[7] Some studies have demonstrated decreased activity of T and B lymphocytes, neutrophils, macrophages, and complement. Therefore, patients with β thalassemia major should be regularly screened for immune systems.

Although the relationship between thalassemia infection and thalassemia immunologic markers has been investigated many times, the number of studies investigating the relationship between increased iron load and immunologic status in transfusion-dependent thalassemia (TDT) patients is limited. In our study, we aimed to retrospectively investigate whether there is a relationship between these cells and ferritin levels in patients with β thalassemia major who were previously screened for T, B, and NK cell markers by flow cytometry in our center.

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METHODS

The study included 61 patients with TDT between the ages of 18 and 45 who were followed up in the hematology clinic of our tertiary care center between 2018 and 2023. Patients with primary immunodeficiency and patients with chronic diseases that may lead to secondary immunodeficiency were excluded. Approval for the study was obtained from the Harran University Clinical Researches Ethics Committee with the decision number HRÜ/23.09.02 and date 22.05.2023. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Patients were also evaluated according to whether they had a splenectomy or not, and it was seen that 34 of the patients had a splenectomy. The demographic data of the patients is presented in Table 1.

Table 1. Sociodemographic and clinical characteristics

Variables	n	%
Age		
Mean ± SD	24.83 ± 6.02	
Median (min-max)	23 (18-45)	
Gender		
Female	33	54.1
Male	28	45.9
Splenectomy		
No	27	44.3
Yes	34	55.7
Parameters	**Mean ± SD**	
Ferritin	3150.88 ± 2553.51	
Number of transfusions per year	15.39 ± 1.90	
Saturation of transfer	42.42 ± 14.43	
CD19 B	12.73 ± 5.52	
CD3 T LENF	73.39 ± 9.47	
CD3, CD4 TLENF	58.90 ± 10.78	
CD3, CD8 T LENF	38.72 ± 13.41	
CD4/CD8 T	1.63 ± 0.60	
CD3- CD56+ NK	10.62 ± 5.02	
CD3 HLADR+ T	12.31 ± 11.23	
Number of infections per year	2.85 ± 2.02	

Serum ferritin was measured by the ELISA method. The 12-month mean serum ferritin value and serum transfusion saturation were used to measure iron toxicity and accumulation in patients diagnosed with TDT.[8] For the follow-up of the infection status of the patients, the applications to the infectious diseases department and the patients diagnosed by this department were retrospectively screened, and one-year applications were evaluated.

Quantification of serum immunoglobulins was performed by the radial immunodiffusion technique (Behring turbometer). For the determination of mononuclear cell surface markers, peripheral blood was processed according to standard procedures, labeled with monoclonal antibodies specific for CD4, CD8, CD3, CD19, and CD56, and analyzed by cytofluorometric analysis with a FACScan flow cytometer (Sysmex UF 1600).

Statistical Analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Descriptive statistics are presented as mean ± SD or median (IQR) for continuous variables. The data were analyzed for normal distribution by the Kolmogorov-Smirnov test, and since $p<0.05$, the Mann-Whitney U test, a nonparametric test, was used for pair-wise group comparisons with continuous variables. The relationship between continuous variables was analyzed by the Spearman correlation test. $p<0.05$ was considered statistically significant.

CONCLUSION

The mean age of the 61 patients included in the study was 24.83±6.02 years, and 33 were female and 28 were male. The mean ferritin value was 3150.88±2553.51 ng/ml, and transferrin saturation was 42.42±14.43. The mean number of transfusions per year was 15.39±1.90, and the mean number of detectable infections per year was 2.85±2.02. The sociodemographic and clinical characteristics of the patients are presented in Table 1.

CD3+ levels were measured in all patients, and the mean was 73.39±9.47%. No significant difference was found in CD3+ levels of splenectomized patients compared to non-splenectomized patients (p=0.36) (Table 2). There was also no significant difference between CD3+ levels and ferritin and transferrin saturation values. There was no significant difference between CD3+ levels and the number of infections per year. CD 3 HLADR+T cells were significantly higher in patients who underwent splenectomy (p=0.014) and CD 3 HLADR+T cells were increased in patients with frequent infections, but this was not statistically significant (p=0.144) (Table 3).

Table 2. Comparison of various clinical parameters with splenectomy

	Splenectomy		
Variables	**No N=27 Median (IQR)**	**Yes N=34 Median (IQR)**	**p**
CD19 B	12.0 (6.0)	12.0 (10.5)	0.721
CD3 T LENF	75.0 (11.0)	72.5 (11.5)	0.360
CD3,CD4 TLENF	60.0 (19.0)	57.0 (12.2)	0.760
CD3,CD8 T LENF	39.0 (21.0)	35.0 (12.7)	0.416
CD4/CD8 T	1.3 (1.1)	1.6 (0.8)	0.576
CD3- CD56+ NK	10.0 (8.0)	10.0 (6.5)	0.457
CD3 HLADR+ T	8.0 (8.0)	12.0 (10.5)	**0.014**

Mann whitney U test, p<0.05 statistically significant

Helper T cells were measured in all patients, and the mean value was 58.90±10.78%. There was no significant difference in CD4+ levels in splenectomized patients compared to non-splenectomized patients (p=0.76). There was also no significant difference between CD4+ levels and ferritin and transferrin saturation values (p=0.5 and p=0.49, respectively). There was also no significant difference between CD4+ levels and the number of infections seen in one year (p=0.8) (Table 3).

The mean cytotoxic T cell levels were 38.72±13.41% in all patients, and no significant difference was found between the splenectomy and non-splenectomy groups (p=0.41) (Table 2). In addition, no significant difference was found between CD8+

levels and ferritin (p=0.73), transferrin saturation (p=0.83) and the number of detectable annual infections (p=0.96).

The CD4/CD8/CD8 ell ratio was found to be in the normal range, with a mean of 1.63±0.60. This value was 1.3 in non-splenectomized patients and 1.6 in splenectomized patients, and no statistically significant difference was found (p=0.57). There was no significant difference in CD4/CD8 T cell ratio with ferritin (p=0.58), transferrin saturation (p=0.75) and the number of detectable infections per year (p=0.96).

CD56+ natural killer (NK) cells were evaluated in the study, and the mean value was 10.62±5.02%. CD56+ values were statistically similar in the splenectomized and non-splenectomized groups (p=0.45). In addition, NK cells did not change with ferritin value (p=0.34), transferrin saturation (p=0.25) and number of infections (p=0.91).

Finally, B lymphocyte marker CD19+ values were also evaluated in the study, and the mean value was 12.73±5.52 %. CD19+ values were statistically similar in the splenectomized and non-splenectomized groups (p=0.71). There was no significant difference between CD19+ value and ferritin (p=0.48), transferrin saturation (p=0.86), but CD19+ value increased as the annual number of detectable infections increased, but this increase was not statistically significant (p=0.2).

Table 3. Relationship between various clinical parameters

		Ferritin	Saturation of transfer	Number of infections per year
CD19 B	r	0.091	-0.022	0.165
	p	0.483	0.866	0.205
CD3 T LENF	r	-0.182	-0.040	-0.196
	p	0.161	0.758	0.130
CD3,CD4 TLENF	r	-0.087	-0.090	-0.033
	p	0.503	0.491	0.803
CD3,CD8 T LENF	r	-0.027	-0.045	-0.006
	p	0.834	0.730	0.965
CD4/CD8 T	r	-0.071	-0.040	-0.006
	p	0.588	0.757	0.961
CD3- CD56 + NK	r	-0.123	-0.147	0.015
	p	0.346	0.257	0.911
CD3 HLADR + T	r	0.016	-0.014	0.189
	p	0.901	0.914	0.144

Spearman correlation test

DISCUSSION

Infectious complications, which are associated with morbidity and mortality in patients with TDT, constitute an important part of the clinical spectrum of β-thalassemia.[9] In our study, CD4, CD8, CD3, CD19, and CD56 values, which are lymphocyte markers, were examined to evaluate the immune system of patients diagnosed with TDT, and it was observed that there was no significant correlation between these values and the annual infection frequency and mean ferritin values of the patients.

In the study by Ezer et al.[10] lymphocyte subgroups of 27 children diagnosed with thalassemia major were examined. In this study, 15 of 27 patients were splenectomized, and unlike our study, 12 patients diagnosed with the thalassemia trait were included in the study, and a control group was added to the study. Again, unlike our study, the CD8+ level was found to be significantly higher in the non-splenectomized group in this study. In addition, CD19+ levels were also found to be significantly higher in patients diagnosed with thalassemia in this study. In this study, there was no comparison between ferritin value and lymphocyte subgroups in children diagnosed with thalassemia.

In the study by Rakhmanova et al.[11], CD4, CD8, CD3, and CD16 levels as immune markers, IL-6 and IFN-β values, and ferritin values were examined in 200 patients aged between 1 and 27 years. It was not specified how many of the patients were in the adult age range. In this study, the CD3+ cell ratio was found to be high at 39.5%, but unlike our study, immune markers and lymphocyte subgroups were not compared with splenectomy and ferritin levels. However, the fact that CD3 HLADR+ T cells were found to be high, especially in patients with splenectomy, in our study may be considered a similarity between the two studies.

In the study conducted by Ehsanipour et al.[12] in 106 patients aged between 4 and 50 years, many parameters, including complement levels, lymphocyte markers, immunoglobulin levels, use of chelator drugs, ferritin, and age, were examined. Similar to our study, lymphocyte markers were also examined in patients, and similar to our study, no significant correlation was found between splenectomy, ferritin levels, and lymphocyte markers. Unlike our study, the adult age group was not evaluated separately in this study, and the relationship between the frequency of infection and lymphocyte sub-markers was not investigated.

The number of studies evaluating the relationship between the immune system and ferritin levels in patients with TBI in the adult age group is limited. In our study, we contributed to this deficiency in the literature. The biggest shortcoming of our study is that it was conducted at a single center. Studies involving a larger number of centers and patients are needed to confirm the relationship between ferritin, which is an indicator of iron load, and the immune system in patients with TBI.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ethical Committe of Faculty of Harran University (Date: 22.05.2023, Decision No: HRÜ/23.09.02).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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